PROJECT NUMBER: 6906
PROJECT TITLE: Biological Effects of Smoke
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I. INHIBITION OF EGF BINDING

A. Objective: Establish the EGF assay and determine the effects of CSC on EGF binding.

B. Results: An experiment was conducted this month to determine the quantitative properties of this assay by testing selected CSC samples. The cigarette samples tested were Kentucky Reference 2R1, the 100% bright expanded stems, and 75% DBC burley-25% bright expanded stems. The results indicated that all CSC samples exhibited equal growth inhibitory effects in a dose-dependent manner. Analysis of the binding data indicated that all CSCs inhibited EGF binding. Preliminary analysis suggests that that there was no difference in activity between the CSCs tested, as the assay is currently being conducted. One interesting observation in this experiment was that the solvent control (DMSO) also significantly inhibited EGF binding. This finding could have profound effects on the degree of activity of the CSCs tested.

C. Plans: Eliminate the effects of the solvent control on EGF binding.

D. References:

Stagg, D. L. Notebook 8553, pp. 49-64.

II. MAINTENANCE OF 3T3 CELL LINE

A. Objective: Evaluate a new low passage 3T3 cell line in the EGF assay.

B. Results: Because of extensive use of the routine 3T3 cell cultures (passage #224), it was decided to evaluate a new low passage 3T3 cell line. Both cell lines were tested in head-to-head experiments to the determine normal EGF binding and the inhibitory effects of CSC on EGF binding. The results indicated that the new and old cell line produced similar values with regard to EGF binding and inhibition of binding with CSC. The early passage cells will now be routinely used in all future experiments.

C. References:

Patskan, G. J. Notebook No. 8527 pp. 87-134.

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III. GLUTATHIONE DEPLETION ASSAY (GDA)

A. **Objective**: Evaluate the CSC activity from washed shredded bright stem (WSBS) cigarettes having whistle through (WT), charcoal, THAM on charcoal, and basic alumina filters.

B. **Results**: The WSBS CSCs from cigarettes with WT, charcoal, THAM on charcoal, and basic alumina filters were tested to determine what effect filters (designed to remove aldehydes) would have on GDA activity.

Previous gas phase and whole smoke studies indicated that these filters reduced GDA activity. The results obtained from this experiment were surprising in that the CSCs from the charcoal and THAM on charcoal cigarettes were more active than the control WT CSC. The reason(s) for this finding is now under discussion.

C. **Plans**: Test CSCs from cigarettes made with these filters and 100% DBC burley filler.

D. **References**:

McCoy, W. R. Notebook No. 8555, pp. 16-17.

IV. ACYLARACHIDONYL GLYCEROL (AAG) IN 3T3 CELLS

A. **Objective**: To isolate ^{3}H-AAG from 3T3 cells.

B. **Results**: Thin layer chromatography of lipids extracted from cells grown on permanox (plastic) plates did not show a region of radioactivity corresponding to AAG. Re-chromatographing the sample in a different solvent system indicated that the majority of the ^{3}H was incorporated into phospholipids. A second experiment using cells grown on glass vs. permanox still did not show ^{3}H-AAG on TLC (1). In a third experiment, the amount of sample spotted on TLC was greatly increased, and preliminary indications are that concentration of the sample is a factor, because there was radioactivity detected in the region corresponding to AAG (2).

C. **Plans**: Develop experimental procedures to concentrate and quantitate lipid extracts from 3T3 cells.

D. **References**:

Nixon, G. Notebook No. 7758, pp. 165-168, 173-176.
Nixon, G. Notebook No. 8569, pp. 1-4, 7-8.

V. SALMONELLA/MICROSOME (S/M) ASSAY

A. **Objective**: To determine if methyl glyoxal could be used as a positive control for TA104.

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B. **Results**: In spite of low titres and high background from experiments with TA104, the strain responded favorably to titres, sensitivity checks, 2R1 CSC and methyl glyoxal as a positive control.

C. **Plans**: Reisolate TA104 strain and evaluate IT CSC's from 7 model CSC cigarettes.

D. **References**:

Coleman, S. L. Notebook No. 8465, pp. 91-93.

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